STUDIES ON THE CILIATES FROM FRESH WATER MUSSELS

II. The Nuclei of Conchophthirius Anodontæ Stein, C. Curtus Engl., and C. Magna Kidder, during Binary Fission

GEORGE W. KIDDER

(From the College of the City of New York and the Marine Biological Laboratory, Woods Hole, Mass.)

In a previous paper (Kidder, 1934) I have given a description of the general morphology, neuromotor apparatus, and vegetative nuclei of the three species of *Conchophthirius* to be discussed in the following pages. Mention was made of the occurrence of binary fission, and the general appearance of the metaphase spindles was figured. It is the purpose of the present paper to report the cytological details of the nuclear phenomena during division and to point out differences from and similarities to the nuclei of related forms.

Relatively few of the many descriptions of ciliate division give a detailed account of the number and behavior, during binary fission, of the micronuclear chromosomes. Especially noticeable is the general lack of close observations as to the period between the metaphase and the anaphase stages with regard to the method of chromosomal division. This situation is quite obviously due to the difficulty of observation of so small a cell element and also in part to the usually short duration of this stage. Whether ciliate chromosomes divide transversely or longitudinally is of great theoretical significance, as was clearly pointed out by Calkins (1930*a*). A great many more observations on this point are needed before we can formulate any general conclusions.

Due to the large size of the micronucleus of *Conchophthirius anodontæ*, the great abundance of material, and the ease of fixing and staining, I have been able to make a detailed study of the chromosomal division in this species. Although the other two species studied, *C. curtus* and *C. magna*, were very plentiful, their micronuclei are so small, as pointed out before (Kidder, 1934), that I was unable to observe the chromosomes clearly and so could not be sure of the exact method of chromosomal division. In all three species the changes occurring in the macronuclei during fission could be followed with ease.

The material used in this study was obtained from the same sources as those previously reported (Kidder, 1934). The preparations were

made at the Marine Biological Laboratory at Woods Hole, Massachusetts.

Technique

The method of obtaining the ciliates for study of the division process was similar to that used in the study of *Conchophthirius mytili* (Kidder, 1933). As in that species, the present ciliates can be selected for fixation by observing, through a dissecting binocular, the condition of the macronucleus. In this manner practically any stage can be obtained in a very short time. However, unlike *Conchophthirius mytili,* the present ciliates are so numerous that a single mussel will often yield all of the various stages of binary fission.

The fixatives employed were Flemming's, Schaudinn's, Bouin's, and Gilson-Carnoy's fluids and sublimate-acetic in 95 per cent alcohol. Flemming's fluid was followed by bleaching in H_2O_2.

The stains giving uniformly good results were Heidenhain's and Delafield's hæmatoxylins, the Borrel stain and the Feulgen thymonucleic acid reaction. By far the best preparations were obtained by the Feulgen reaction following Schaudinn's fluid or sublimate-acetic in 95 per cent alcohol, and Heidenhain's hæmatoxylin (long method) after Schaudinn's or Flemming's fluids.

I employed a modification of the picric acid destaining method of Tuan (1930) following Heidenhain's hæmatoxylin. For this modification I am indebted to Messrs. T. T. Chen and R. Wichterman. After staining, the coverglasses are rinsed in water and placed in a saturated aqueous solution of picric acid. Usually twenty to thirty minutes are required to extract a sufficient amount of stain. Critical examination can be made at any time during the destaining. By passing the coverslip over the mouth of a bottle containing concentrated ammonium hydroxide the brownish appearance imparted by the picric acid is removed. The fumes change the brown of the picric to a bluish black and render the organism quite clear. Care should be taken not to expose the coverslips to the ammonium hydroxide for a longer period than is needed to produce the bluish color, as excess will result in the swelling of the organism. When sufficient stain has been extracted, so that the cytoplasm is a pale gray, the coverglasses are washed in running water for thirty minutes, dehydrated and mounted.

Observations

Conchophthirius anodontæ Stein

Micronucleus.—The first sign of division in this ciliate is to be found in the swelling and loss of staining capacity of the micronucleus.

From the compact vegetative condition the chromatin becomes flocculent and the nuclear membrane moves away from the central mass leaving a clear area. This clear area is no doubt partly the result of shrinkage brought about by fixation. The micronucleus moves out of its pocket in the macronucleus and takes up a position in the mid-region of the cell. The chromatin becomes irregularly disposed in a reticulate fashion (Fig. 1, *A*). Swelling continues and the chromatin condenses into a twisted band, which at first is quite irregular. This is similar to a spireme stage. (Fig. 1, *B*). I am unable to say whether the spireme band is continuous or broken at this time. In the clear space at the poles of the chromatin mass delicate spindle fibers can be seen. These fibers appear to be pushing out from the central matrix. The spireme band becomes more basophilic and breaks into many small segments. These segments orient themselves along the long axis of the nucleus, with their ends pointing toward the rapidly forming spindle fibers (Fig. 1, *C*). The whole nucleus is undergoing elongation. The chromatin making up the bands is disposed in irregularly spherical chromomeres of considerable size. In carefully differentiated material it is possible to count these bands even at this early stage. The number is twelve. The metaphase plate is formed by a condensation of the twelve bands into definite but rough chromosomes, a concomitant elongation of the nucleus, and completion of the division spindle (Fig. 1, *D*). The chromosomes of the metaphase plate are always lined up along the long axis of the spindle.

I have never seen any evidence of an endosome at any stage in the micronuclear mitosis, such as was found by Wenrich (1926), Manwell (1928) and Turner (1930).

About this time the first indication of actual chromosomal division may be seen. A longitudinal split occurs in each chromosome and eventually the two daughter chromosomes are clearly discernible (Fig. 1, *E*). Material stained after the Feulgen reaction was the most satisfactory for this stage as the chromosomes are sharply outlined and there is none of the haziness so often seen in material stained in hæmatoxylin. Unlike the majority of ciliates, the metaphase appears to be of rather long duration and dozens of well-prepared individuals were obtained for study. In no case, however, was I able to detect the split in all the chromosomes at the same time. The metaphase plate is quite regular and all the chromosomes are of approximately equal length. The migration of the daughter chromosomes to their respective poles is again a relatively slow process, judging from the great number of anaphases obtained. The daughter halves slip by one another resulting in a broad band of chromosomes in the central portion of the spindle. Each daughter increases somewhat in size. It is possible at all times during

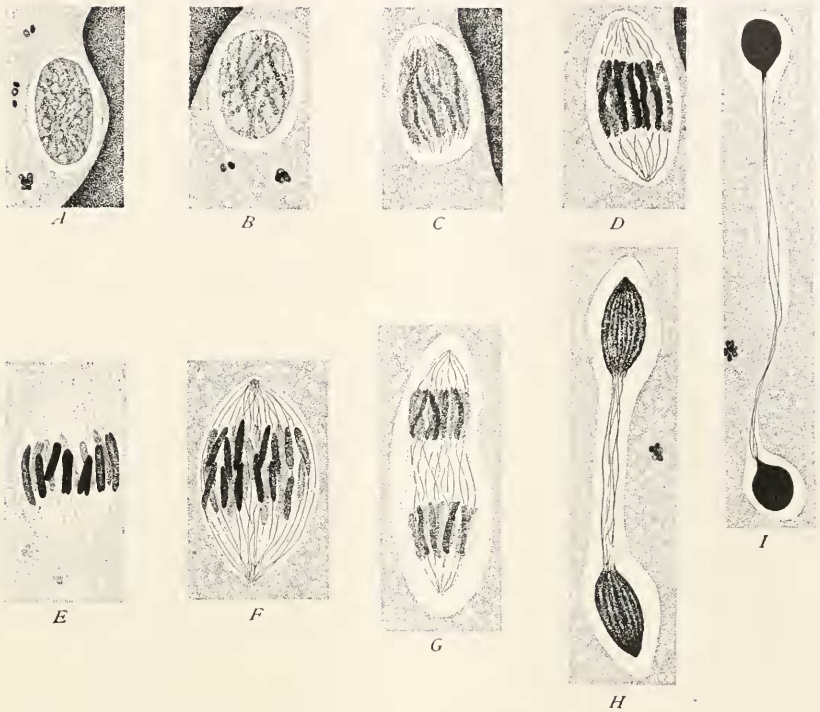

FIG. 1. The micronucleus of *Conchophthirius anodontæ* during mitosis. All except *E* were fixed in Schaudinn's fluid, stained with Heidenhain's hæmatoxylin and destained in aqueous picric acid. *E* was fixed in sublimate acetic in 95 per cent alcohol and stained after the Feulgen reaction. Drawings were made with the aid of a Promar projector. × 2644.

A. Early prophase with the chromatin in the flocculent state.

B. Spireme stage. The spindle fibers are just beginning to appear.

C. Late prophase. Twelve long strands are oriented toward the poles of the forming spindle.

D. Early metaphase. Twelve chromosomes.

E. Metaphase. Twelve fully contracted chromosomes. Some show definite longitudinal splits while in others only the notched condition of the ends indicates where the split will appear.

F. Early anaphase. Daughter chromosomes migrating towards their respective poles.

G. Late anaphase. The two groups of twelve chromosomes each are well separated.

H. Early telophase.

I. Late telophase. Separation spindle clearly shown.

this slipping-past process to count the twenty-four daughter halves (Fig. 1, *F*). Not only is it possible to make consistent counts but because of their proximity it is usually possible to detect which two halves were originally a single chromosome. The metaphase split and the early anaphase migration are undoubtedly the most interesting features of this extremely large and clear micronucleus. I have never seen any indication of the dumb-bell-shaped chromosomes of the early anaphase that occur so frequently in ciliates, and because of the extreme numbers of all stages of mitosis available for study, the large size, and the clear staining properties of this micronucleus, I feel certain that the activity described above is accurate as far as *Conchophthirius anodontæ* is concerned.

The late anaphase is quite regular. Twelve daughter chromosomes migrate to the poles of the now elongated spindle (Fig. 1, *G*). Here they contract into a typical spear-head mass. The fibers of the spindle retain their form between the two daughter nuclei (Fig. 1, *H*) even to the very late telophase. The formation of the daughter nuclei is regular, the chromatin contracting into two homogeneous spheres (Fig. 1, *I*). As the daughter nuclei move apart a long " separation spindle " is pulled out between them. In the flared portion of the separation spindle a number of fibers persist for some time. The separation spindle and contained fibers gradually fade out and the two micronuclei round up and take up their positions against the daughter macronuclei.

Macronucleus.—During the early prophases of the micronucleus, the macronucleus of *Conchophthirius anodontæ* swells slightly and, as the late prophase of the micronucleus sets in, migrates to a central position in the cell. During the metaphase of the micronucleus, the macronucleus elongates in the direction of the long axis of the cell and internal changes take place. The chromatin becomes evenly granular and as constriction starts an area of more deeply staining chromatin forms in the central region. This island is marked off from the major portion of the macronucleus by a halo of less deeply staining chromatin (Fig. 2, *A*). The differentiation of the central area is not the same in all individuals, for in some the nuclear granules appear evenly distributed until a later stage. The central mass is the residual chromatin characteristic of the Conchophthiriidæ. The residual mass condenses into a densely staining sphere between the dividing daughter halves of the macronucleus (Fig. 2, *B*). The macronuclear membrane flares out to accommodate the residual mass just as it does in *Conchophthirius mytili* (Kidder, 1933*a*) and *Ancistruma isseli* (Kidder, 1933*b*). Subsequent pulling apart of the daughter halves leaves the residual mass near the cell center (Fig. 2, *C*). Finally the connections break and the residual mass rounds up

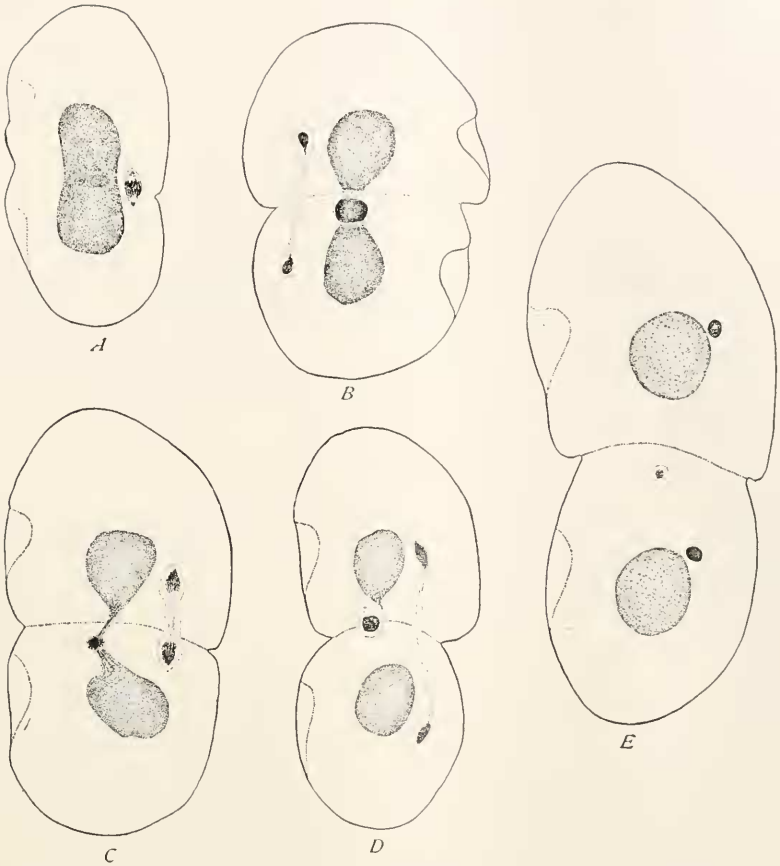

FIG. 2. The macronucleus of *Conchophthirius anodontæ* during binary fission. Camera lucida drawings. × 511.

A. Earliest evidence of the formation of the ball of residual chromatin. Gilson-Carnoy's fluid—Heidenhain's hæmatoxylin.

B. Residual ball somewhat contracted and lying between the separating halves of the macronucleus. Schaudinn's fluid—Heidenhain's hæmatoxylin.

C. Further contraction of the residual ball and separation of the macronuclear halves. Schaudinn's fluid—Heidenhain's hæmatoxylin.

D. Daughter macronuclei have separated. Residual ball has rounded up within a fragment of the old macronuclear membrane. Gilson-Carnoy's fluid—Delafield's hæmatoxylin.

E. Plasmotomy well under way. Residual chromatin is fading out in the cytoplasm. Sublimate acetic—Feulgen reaction.

(Fig. 2, *D*). Plasmotomy proceeds and the residual chromatin, in the cytoplasm of either of the daughter ciliates, starts to disintegrate and rapidly loses its affinity for basic stains (Fig. 2, *E*) until finally it becomes completely absorbed. Plasmotomy may or may not be completed before the entire dissolution of the residual chromatin has occurred.

In a few cases of division in this species no residual chromatin was visible, the macronucleus dividing cleanly. These cases were similar to the reorganized exconjugants of *Conchopthirius mytili* (Kidder, 1933*a*). I believe it highly possible that the cases of cleanly dividing macronuclei of *Conchophthirius anodontæ* also represent organisms shortly after conjugation. My belief is based on the presence of considerable numbers of reorganizing individuals found during the first month of this investigation (June, 1933), indicating that conjugation had previously taken place. I will have more to say on this matter in a later paper.

Irregularities of division, which I am inclined to regard as abnormal and probably pathological, are occasionally encountered. A few dividing organisms showed an obvious upset in the normal procedure. In some cases the macronuclear division was very asymmetrical, the greater portion being included in one daughter organism (Fig. 7, *A*). In others the nuclear division failed to keep pace with plasmotomy, all of the chromatin remaining in one daughter, leaving the other devoid of nuclei (Fig. 7, *B*). In still other cases only a small portion of the macronucleus is passed to one daughter ciliate while the other daughter retains most of the macronuclear material and all of the micronuclear material (Fig. 7, *C*). In some of these cases we have visible evidence of the possibility of the formation, through faulty fission, of an amicronucleate race. Whether or not this ever occurs, or indeed whether or not either daughter is viable, I cannot say.

Conchophthirius curtus Engl.

Micronucleus.—The general activity of the micronucleus of *Conchophthirius curtus* is very similar to that of *C. anodontæ* but because of its small size it is impossible to follow the finer details of exact chromosome number and method of chromosomal division, at least in my material.

The early prophase occurs as the micronucleus emerges from its pocket in the plastic macronucleus. It swells slightly, the chromatin becoming loosened and finely granular and less deeply staining than in the vegetative state (Fig. 3, *A*). The micronucleus now elongates,

always in the plane of the long axis of the body of the ciliate. The chromatin becomes condensed into many granular threads, lined up along the long axis (Fig. 3, *B*). These threads contract, increase in staining capacity, and form the chromosomes of the metaphase plate (Fig. 3, *C*). In the meantime spindle fibers have pushed out to form a rather sharply pointed spindle. The chromosomes lie very close to one another and I have not been able to make accurate counts, even after the Feulgen reaction. The next stage is not clear. It is very hard to determine

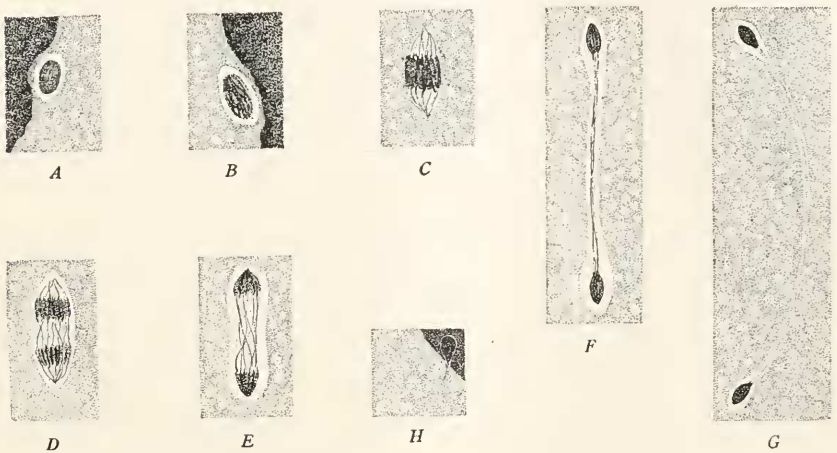

FIG. 3. The micronucleus of *Conchophthirius curtus* during mitosis. Camera lucida drawings. × 2663.

A. Early prophase. Micronucleus migrating from its pocket in the macronucleus. Gilson-Carnoy's fluid—Heidenhain's hæmatoxylin.

B. Prophase. Chromatin in strands. Sublimate acetic—Feulgen reaction.

C. Metaphase. Gilson-Carnoy's fluid—Heidenhain's hæmatoxylin.

D. Anaphase. Schaudinn's fluid—Heidenhain's hæmatoxylin.

E. Early telophase. Schaudinn's fluid—Heidenhain's hæmatoxylin.

F. Telophase. Gilson-Carnoy's fluid—Heidenhain's hæmatoxylin.

G. Later telophase. Schaudinn's fluid—Heidenhain's hæmatoxylin.

H. Reconstruction of one daughter micronucleus. Evidence of separation spindle still seen. Sublimate acetic—Heidenhain's hæmatoxylin.

whether a broad band of chromosomes represents a contraction from the prophase or a migration at early anaphase. Of this I am certain, however, that in the dozens of cases of this stage studied I have never found the dumb-bell-shaped chromosomes such as were found in *Conchophthirius mytili* (see Plate II, Fig. 16 in Kidder, 1933*a*). This would lend support to the view that chromosomal division is longitudinal as in *C. anodontæ,* and that in the early anaphase migration the two daughters slip past one another.

The later anaphase is quite clear. Two groups of evenly distributed chromosomes are seen toward the poles of the spindle (Fig. 3, *D*). Between the two daughter groups of chromosomes the mid-fibers are always quite clear. Further elongation of the micronucleus and further migration of the daughter chromosomes completes the anaphase (Fig. 3, *E*). Very shortly the chromosomes lose their identity in the spearhead telophase chromatin mass (Fig. 3, *F*). These daughter micronuclei pull further and further apart, stretching out an extremely long connecting strand, composed of the mid-fibers and the nuclear membrane (Fig. 3, *G*). It is usually not until the daughter micronuclei have entered the daughter macronuclei that this connection is ruptured. For some time thereafter traces of the connecting strand can be seen extending from the otherwise contracted micronuclei (Fig. 3, *H*).

Macronucleus.—The phenomena accompanying the division of the macronucleus of *Conchophthirius curtus* so closely resembles that of *C. anodontæ* that I shall not dwell at length on this subject.

The furrowed macronucleus migrates into the mid-region of the cell and elongates. A heavily staining ball of chromatin becomes differentiated from the rest of the macronuclear chromatin in the central region. This ball is surrounded by a less densely staining halo (Fig. 4, *A*). As the macronucleus constricts the ball contracts (Fig. 4, *B*) and remains between the daughter macronuclei (Fig. 4, *C*). The residual mass retains its connections with the daughter macronuclei for some time (Fig. 4, *D*). It is finally freed and then rounds up in the cytoplasm of either daughter ciliate (Fig. 4, *E*). The daughter macronuclei lose their smoothly granular condition and again become furrowed and the residual chromatin disintegrates and disappears.

I have found several cases where no residual chromatin was formed, the macronuclei separating cleanly. As in *C. anodontæ* and *C. mytili* I am inclined to regard these cases as ciliates shortly after exconjugant reorganization. I have found numerous conjugating pairs and many reorganizing individuals, and the cases in which a residual mass was lacking were in about the proportions one might expect if these cases do represent exconjugants. Future investigation on the conjugation of these ciliates may shed more light on this interesting problem.

Abnormalities of fission were rarely encountered in this species. Three cases of precocious plasmotomy were observed where the macronucleus and the micronucleus were included in a single daughter, the other daughter being entirely devoid of nuclear material (Fig. 7, *D*).

Conchophthirius magna Kidder

Micronucleus.—As I have previously stated (Kidder, 1934), *Conchophthirius magna* possesses, in about 90 per cent of the cases, two small,

compact vegetative micronuclei. Dividing individuals, in which the micronuclei are quite easily seen, confirmed this percentage. About 10 per cent of the individuals possess but one micronucleus. In two cases,

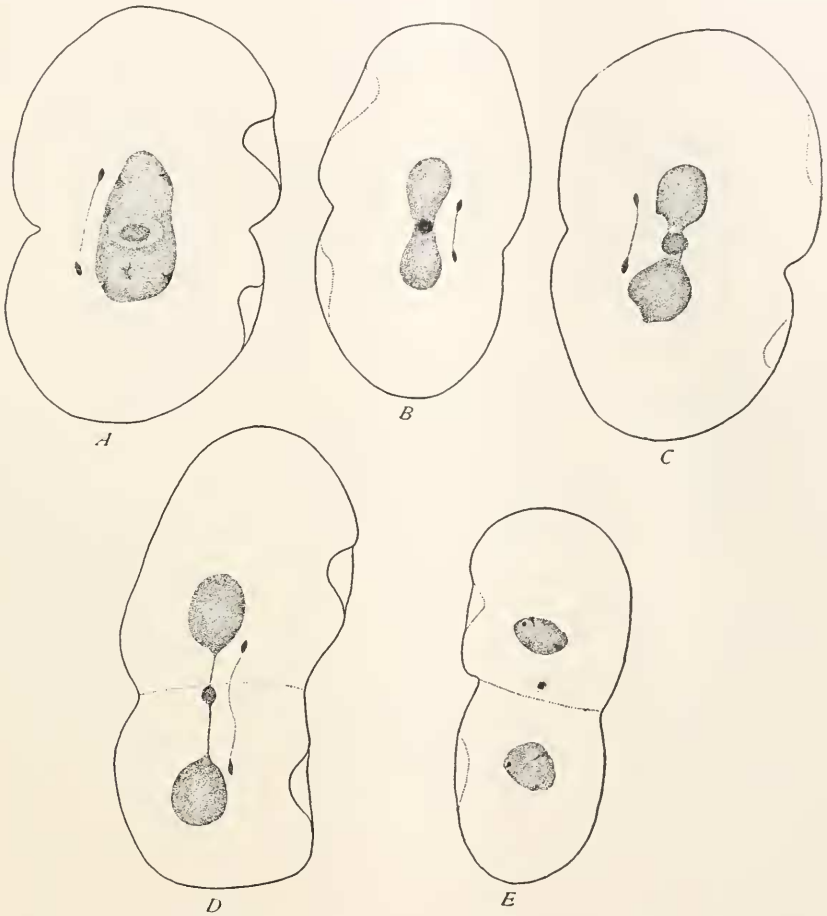

FIG. 4. The macronucleus of *Conchophthirius curtus* during binary fission. Camera lucida drawings. × 521.

A. Formation of the ball of residual chromatin. The macronucleus is still in a furrowed condition. Schaudinn's fluid—Heidenhain's hæmatoxylin.

B. Constriction of macronucleus. Residual chromatin quite compact. Gilson-Carnoy's fluid—Heidenhain's hæmatoxylin.

C. Further constriction of the macronucleus and separation of the residual chromatin. Flemming's fluid—Heidenhain's hæmatoxylin.

D. Residual chromatin connected to daughter macronuclei. Gilson-Carnoy's fluid—Heidenhain's hæmatoxylin.

E. Daughter macronuclei reorganized. Residual chromatin disintegrating. Sublimate acetic—Feulgen reaction.

however, I was unable to see any micronuclei. These individuals, though in the early stages of fission, contained an enormous amount of food which, I believe, obscured the spindle or spindles that may have been present.

The micronuclei of *C. magna* appear to possess a greater tendency to remain near or in contact with the macronucleus during the prophase, metaphase and anaphase stages than do those of *C. anodontæ* and *C.*

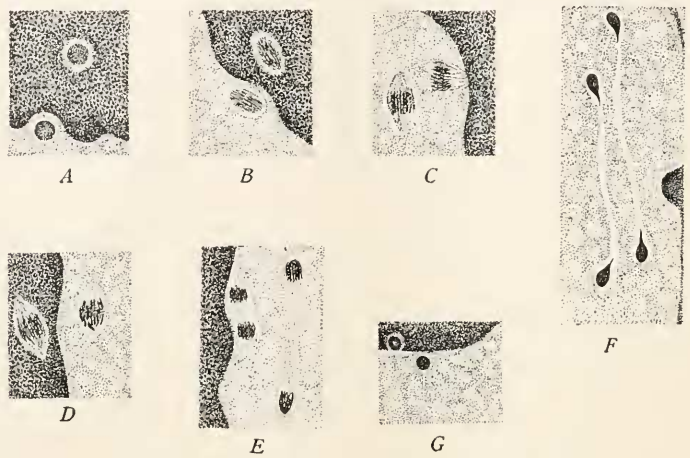

FIG. 5. The micronuclei of *Conchophthirius magna* during mitosis. Camera lucida drawings. × 2076.

A. Early prophase. Gilson-Carnoy's fluid—Feulgen reaction.
B. Late prophase. Sublimate acetic—Heidenhain's hæmatoxylin.
C. Metaphase. Sublimate acetic—Feulgen reaction.
D. Early anaphase. Schaudinn's fluid—Feulgen reaction.
E. One micronucleus in middle anaphase and one in late anaphase. Gilson-Carnoy's fluid—Heidenhain's hæmatoxylin.
F. Telophase. Gilson-Carnoy's fluid—Feulgen reaction (counterstained in Borrel II).
G. Late telophase. The micronuclei are compact and spherical but still connected to the long separation spindles. Schaudinn's fluid—Heidenhain's hæmatoxylin.

curtus. Swelling of the micronuclei results in a finely granular condition at the onset of mitosis (Fig. 5, *A*). The chromatin becomes oriented into granular threads extending nearly the entire length of the somewhat elongated nucleus (Fig. 5, *B*). These threads contract and form rather definite chromosomes on the equatorial plate of the now fully formed spindle (Fig. 5, *C*). These chromosomes are again too small and too compact to enable one to count them. The beginning of the anaphase is again, as in *C. curtus*, a question of interpretation. I believe Fig. 5, *D*

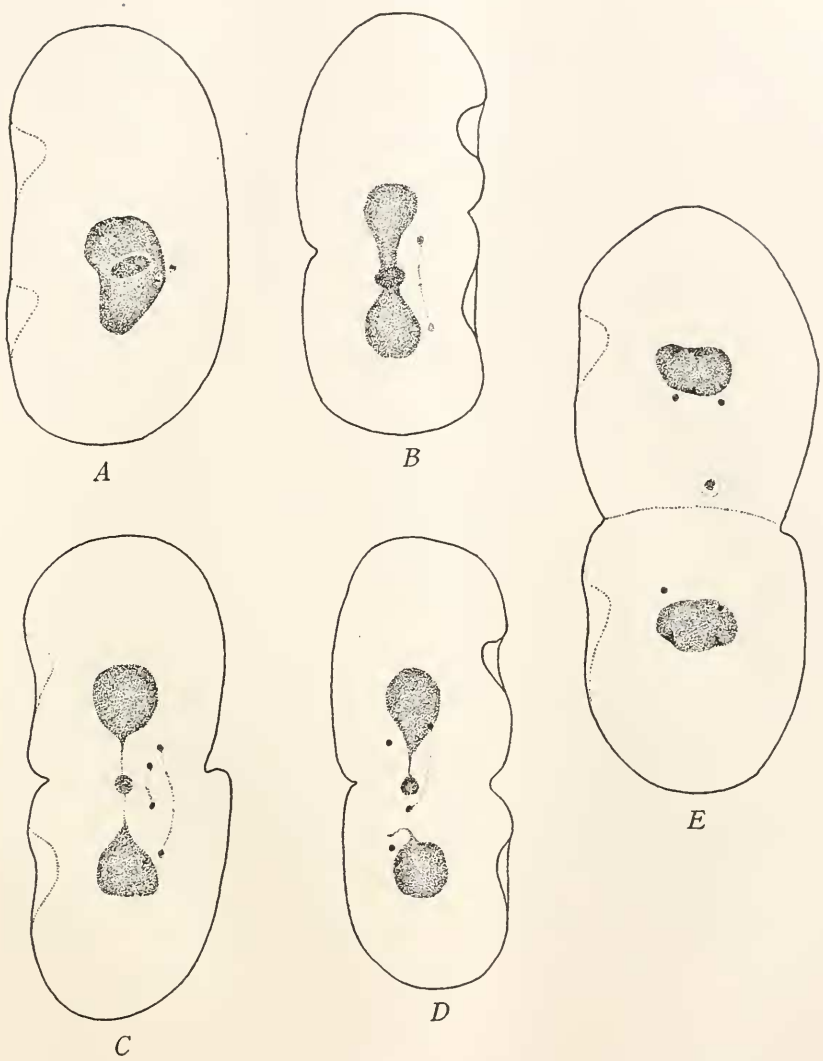

FIG. 6. The macronucleus of *Conchophthirius magna* during binary fission. Individuals were all fixed in sublimate acetic in 95 per cent alcohol and, with the exception of *E*, were stained after the Feulgen reaction. *E* was stained in Heidenhain's hæmatoxylin. Camera lucida drawings. × 350.

A. Formation of ball of residual chromatin.

B. Constriction of macronucleus and separation of residual ball. The individual possessed a single micronucleus.

C. Separation of daughter macronuclei.

D. Separation of one daughter macronucleus from residual ball.

E. Reorganization of daughter macronuclei and disintegration of residual chromatin.

represents the slipping past of daughter chromosomes on their way to their respective poles. I have never seen any dumb-bell-shaped chromosomes that would indicate transverse division.

The later anaphases are quite regular and very similar to those of *C. curtus* (Fig. 5, *E*). The mid-fibers are clearly demonstrated between the two daughter groups of chromosomes. In the telophase the daughter

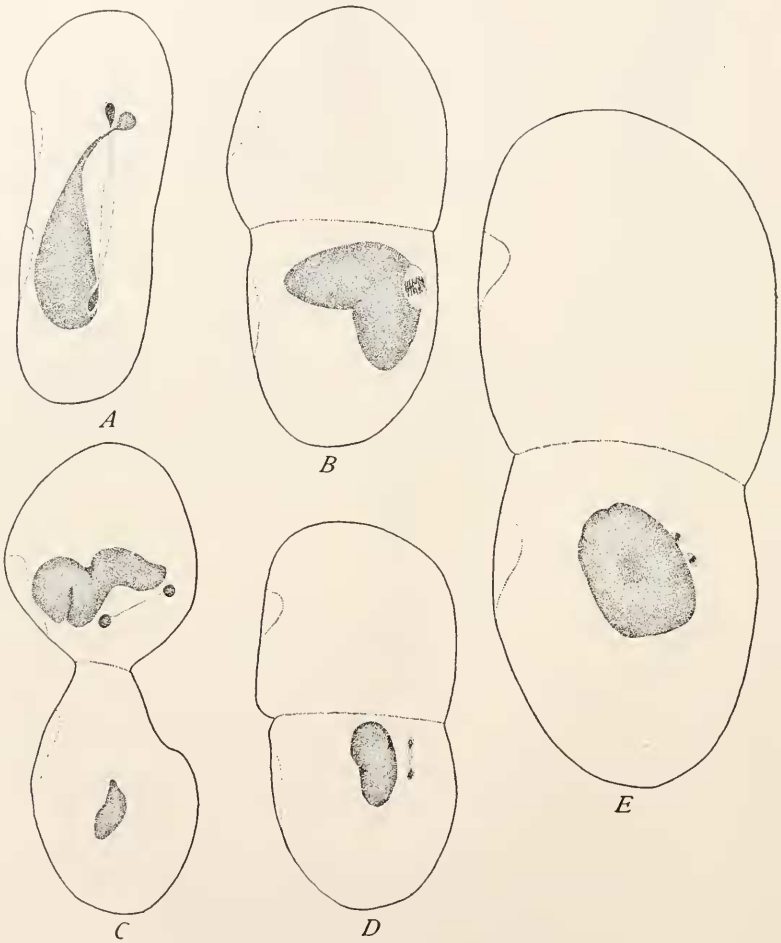

FIG. 7. Abnormal divisions. × 486.

A. Conchophthirius anodontæ. Schaudinn's fluid—Feulgen reaction.
B. C. anodontæ. Gilson-Carnoy's fluid—Heidenhain's hæmatoxylin.
C. C. anodontæ. Gilson-Carnoy's fluid—Heidenhain's hæmatoxylin.
D. C. curtus. Sublimate acetic—Heidenhain's hæmatoxylin.
E. C. magna. Bouin's fluid—Heidenhain's hæmatoxylin.

micronuclei pull out a long connecting strand (Fig. 5, *F*) which, in some cases, persists until the four compact daughter micronuclei enter the macronuclei (Fig. 5, *G*).

Macronucleus.—As the micronuclei prepare for mitosis the macronucleus of *Conchophthirius magna* begins to lose its furrowed condition and finally becomes evenly granular. A large mass of deeply staining chromatin, surrounded by a lightly staining halo, is differentiated near its center (Fig. 6, *A*). This mass contracts into a ball and is left in the division plane as the daughter halves of the macronucleus pull apart (Fig. 6, *B*). Long connecting strands persist for some time (Fig. 6, *C*) as in the preceding species. When these strands are severed it is a common occurrence to find one or both curved back on itself (Fig. 6, *D*) as if they had been suddenly released from a stretching force. As the daughter macronuclei become reorganized in the daughter ciliates the residual chromatin rounds up and degenerates (Fig. 6, *E*), being absorbed into the cytoplasm of that daughter cell in which it lies.

These phenomena exactly parallel those of *Conchophthirius anodontæ* and *C. curtus*.

So far I have never encountered dividing specimens of *C. magna* lacking the residual chromatin, nor have I ever seen any indications of conjugation, either conjugating pairs or reorganizing individuals. This is evidence, I believe, for my above view, that the lack of the residual chromatin in these forms is in some way connected with the reorganization of the macronuclear chromatin following conjugation, a view more firmly substantiated in the case of *C. mytili* (Kidder, 1933*a*).

A very few abnormal divisions were encountered where the nuclear apparatus lagged in its division behind the rest of the cell (Fig. 7, *E*). In these cases the spindles and macronuclei appeared normal, except for their position in relation to the division plane, while cytoplasmic reorganization was far in advance.

Discussion

In reviewing the literature on micronuclear mitosis in ciliates one is at a loss to decide just what type of chromosomal division takes place in the majority of forms. It would seem that either transverse or longitudinal occurs even in closely related species. Of course if one is to accept the contention of Calkins (1930*a*) that within the protozoan nucleus there is only one type of gene per chromosome, then this situation would lose its significance. As Calkins points out, it would then be immaterial whether the chromosomes divided longitudinally or transversely.

To review briefly a few of the cases on record one must note that Stevens (1903) says that the four vegetative chromosomes in the micronucleus of *Boveria subcylindrica* divide transversely. This statement is repeated in a later paper (Stevens, 1910). Her figures indicate a pinching in two in the central portion of each long chromosome.

Calkins (1919) says that the vegetative chromosomes of *Uroleptus mobilis* line up with their long axes parallel to the long axis of the spindle. His figures clearly show this condition. But as to the actual division he says "Whether these rods are divided transversely or longitudinally cannot be determined owing to their minute size and densely packed condition" (p. 306).

In the case of *Uroleptus halseyi* the micronuclei are extremely large and, according to Calkins (1930*a*), the vegetative chromosomes divide in a transverse manner. His figures do not show the actual fission of the chromosomes, however, but merely the metaphase plate and later the two anaphase groups.

In *Conchophthirius mytili* (Kidder, 1933*a*) the sixteen well-formed vegetative chromosomes pull out into dumb-bell-shaped bodies at early anaphase and it certainly appears that division could only have been accomplished in a transverse manner.

Turner (1930) figures the eight vegetative chromosomes of *Euplotes patella* in the early anaphase as dumb-bell-shaped bodies. He says "Whether the chromosomes divide longitudinally or transversely has not been definitely established. Their appearance in the early anaphase would indicate that the latter case were true. As the two halves of a chromosome separate, there seems to be a fine connection between them for a short time which soon breaks. This observation is evidence against, but does not exclude the possibility that the chromosomes split longitudinally. The fact that these chromosomes are never seen in any other position than parallel to the long axis of the spindle indicates that if they do split longitudinally the daughter halves must slip past each other rather than that one or both revolves on the end of the other" (p. 210). This last observation is very interesting in the light of conditions here described for *Conchophthirius anodontæ* where an actual slipping past is demonstrated. Turner's prediction was, no doubt, influenced by his finding of the daughter chromosomes slipping past one another in the third maturation division of *E. patella*.

Manwell (1928) states that the vegetative chromosomes of *Pleurotricha lanceolata* appear to split longitudinally and, in the early anaphase, draw out into V-shaped bodies with connecting bands. His figures indicate only the V-shaped chromosomes in the process of migration. No figure of the actual split is given.

One clear case of longitudinal chromosomal division is given by Chen (1932) in a preliminary report on the mitosis in *Zellerielia*. Here the chromosomes form a rather irregular metaphase plate and plainly divide throughout their length. He states " . . . the longitudinal split of the chromosomes shows clearly and the chromatids or daughter halves of each chromosome can be identified " (p. 270). This species, however, is a member of the Opalinidæ and does not possess the typical dimorphic nuclei of the majority of ciliates and it is, therefore, a comparison of questionable value.

From the above citations it is evident that as far as we know at present the micronuclear chromosomes of ciliates may divide either transversely or longitudinally. In the former method the chromosomes pull into dumb-bell-shaped bodies; in the latter no such dumb-bells are formed.

The chromosomes of *Conchophthirius anodontæ,* and perhaps those of *C. curtus* and *C. magna,* fall into the latter category. The longitudinal split and the slipping past of the daughter halves in the early anaphase have been encountered too frequently in my material to allow for an alternative interpretation. Only future investigations of ciliates possessing large micronuclei with relatively few chromosomes will determine how widespread is this method of chromosomal division.

It is becoming more and more apparent that the macronuclear chromatin is intimately bound up in the reorganization process of the ciliate cell, not only after conjugation and endomixis but during and after binary fission. As regards the extrusion of macronuclear chromatin into the cytoplasm my observations on the three species of *Conchophthirius* described above are parallel to those of Behrend (1916) on *Loxocephalus;* MacLennan and Connell (1931) on *Eupoterion pernix* (figured but not described); Kidder (1933*a*) (1933*b*) on *Conchophthirius mytili* and *Ancistruma isseli;* and recently Haas (1933) on the divisions within the cyst of *Ichthyophthirius multifiliis.* Rossolimo and Jakimowitsch (1929) describe the casting out of granular masses from the seven macronuclei of *Conchophthirius steenstrupii* during binary fission. It seems probable that these masses represent extrusion chromatin although the authors place a rather different interpretation upon them. Their conclusions are based solely upon the staining reactions of these masses after the use of iron hæmatoxylin.

I am inclined to believe that the extrusion chromatin, regularly given off from the macronuclei of the above forms, must represent waste substances of prolonged cell metabolism. Calkins (1930*b*) called the regular extrusion of chromatin from the macronuclei of *Uroleptus halseyi* prior to fission a " purification process." So regular and widespread a

phenomenon certainly cannot be without meaning, and it seems possible that the reorganization process that takes place in the macronuclei of the hypotrichous ciliates is merely a different method of accomplishing the same end—the elimination of worn-out substances.

Summary

1. The nuclear phenomena incident to fission are described for three ciliate commensals of fresh water mussels, *Conchophthirius anodontæ, C. curtus,* and *C. magna.*

2. The micronuclear chromatin of *C. anodontæ* forms a granular spireme in the prophase. From this spireme form twelve distinct rod-like chromosomes. On the metaphase plate each chromosome splits longitudinally. In the early anaphase the daughter halves slip past one another and form two groups of twelve chromosomes each. The late anaphase and the telophase are quite regular, the compact daughter micronuclei forming from the twelve daughter chromosomes.

3. The macronucleus of *C. anodontæ* undergoes fission, throwing out a deeply staining ball of chromatin near the division plane. This residual chromatin disintegrates and is absorbed into the cytoplasm.

4. *C. curtus* and *C. magna* parallel *C. anodontæ* in all nuclear activity during fission. The micronuclei are, however, too small to permit the observation of minute details.

5. Several cases of abnormal divisions are reported, occurring in all three species. In these cases it would appear that the synchronization of cytoplasmic and nuclear activity had, in some manner, become disorganized.

6. A short review of the literature dealing with ciliate fission, particularly that concerning micronuclear mitosis and the extrusion of macronuclear chromatin, is given.

LITERATURE CITED

Behrend, Kurt, 1916. Zur Conjugation von Loxocephalus. *Arch. f. Protist.,* **37**: 1.

Calkins, G. N., 1919. Uroleptus mobilis Engelm. I. History of the nuclei during division and conjugation. *Jour. Exper. Zoöl.,* **27**: 293.

Calkins, G. N., 1930*a*. Uroleptus Halseyi Calkins. III. The kinetic elements and the micronucleus. *Arch. f. Protist.,* **72**: 49.

Calkins, G. N., 1930*b*. Uroleptus Halseyi Calkins. II. The origin and fate of the macronuclear chromatin. *Arch. f. Protist.,* **69**: 151.

Chen, T. T., 1932. Nuclear Structure and Mitosis in Zelleriella (Opalinidæ). *The Collecting Net,* **7**: 270.

Haas, Georg, 1933. Beiträge zur Kenntnis der Cytologie von Ichthyophthirius multifiliis Fouq. *Arch. f. Protist.,* **81**: 88.

Kidder, George W., 1933*a*. Studies on Conchophthirius mytili DeMorgan. I. Morphology and division. *Arch. f. Protist.,* **79**: 1.

KIDDER, GEORGE W., 1933*b*. On the Genus Ancistruma Strand (Ancistrum Maupas). I. The structure and division of A. mytili Quenn. and A. isseli Kahl. *Biol. Bull.*, **64**: 1.

KIDDER, GEORGE W., 1934. Studies on the Ciliates from Fresh Water Mussels. I. The structure and neuromotor system of Conchophthirius anodontæ Stein, C. curtus Engl., and C. magna sp. nov. *Biol. Bull.*, **66**: 69.

MACLENNAN, R. F., AND F. H. CONNELL, 1931. The Morphology of Eupoterion pernix, gen. nov., sp. nov. A Holotrichous Ciliate from the Intestine of Acmæa persona Eschscholtz. *Univ. Calif. Publ. Zoöl.*, **36**: 141.

MANWELL, REGINALD D., 1928. Conjugation, Division, and Encystment in Pleurotricha lanceolata. *Biol. Bull.*, **54**: 417.

ROSSOLIMO, L. L., AND FRAU K. JAKIMOWITSCH, 1929. Die Kernteilung bei Conchophthirius steenstrupii St. *Zoöl. Anz.*, **84**: 323.

STEVENS, N. M., 1903. Further Studies on the Ciliate Infusoria, Licnophora and Boveria. *Arch. f. Protist.*, **3**: 1.

STEVENS, N. M., 1910. The Chromosomes and Conjugation in Boveria subcylindrica, var. concharum. *Arch. f. Protist.*, **20**: 126.

TUAN, HSU-CHUAN, 1930. Picric Acid as a Destaining Agent for Iron Hematoxylin. *Stain Technology*, **5**: 135.

TURNER, JOHN P., 1930. Division and Conjugation in Euplotes patella Ehrenberg with Special Reference to the Nuclear Phenomena. *Univ. Calif. Publ. Zoöl.*, **33**: 193.

WENRICH, D. H., 1926. The Structure and Division of Paramæcium trichium Stokes. *Jour. Morph. and Physiol.*, **43**: 81.